

WHAT IS CLAIMED IS:

1. A method for identifying an unknown base sequence present in a target single-stranded nucleic acid comprising the steps of:

5 (a) preparing a probe array in which single-stranded nucleic acid probes of No. 1 to No. n ($n \geq 2$) are arranged as isolated spots on a substrate, the probes each having a base sequence complementary to one of plural base sequences expected to be the unknown

10 base sequence;

15 (b) reacting a single-stranded nucleic acid, which has a base sequence fully complementary to a base sequence of one of the single-stranded nucleic acid probes and is fluorescence-labeled, with the probe array under such conditions that single-stranded nucleic acids complementary to each other form a double-stranded nucleic acid;

removing the unreacted labeled single-stranded nucleic acid, and

20 measuring fluorescence intensity of each spot of the probe array to obtain a first template pattern showing a relationship between location of the probes and fluorescent characteristics;

25 (c) performing the same operation as the step (b) for each of remaining single-stranded nucleic acid probes using a second to a nth single-stranded nucleic acid, and obtaining template patterns of No. 2 to No. n

showing a relationship between location and fluorescent characteristics of the probes;

5 (d) performing the same operation as the step (b) using a sample containing the target single-stranded nucleic acid of unknown base sequence to obtain a sample pattern showing relationship between a position and fluorescent characteristics; and

10 (e) comparing the sample pattern obtained in the step (d) with n pieces of template patterns obtained in the steps (b) and (c), to identify a template pattern showing substantially the same pattern as the sample pattern and identifying the base sequence of the single-stranded nucleic acid used for the preparation of the identified template pattern as the unknown base sequence of the target single-stranded nucleic acid.

2. A method for identifying an unknown base sequence present in a target single-stranded nucleic acid comprising the steps of:

20 (a) preparing a probe array in which single-stranded nucleic acid probes of No. 1 to No. n ($n \geq 2$) are arranged as isolated spots on a substrate, the probes each having a base sequence complementary to one of plural base sequences expected to be the unknown base sequence;

(b) reacting a single-stranded nucleic acid which has a base sequence fully complementary to a base

sequence of one of the single-stranded nucleic acid probes and is fluorescence-labeled, with the probe array under such conditions that single-stranded nucleic acids complementary to each other form a double-stranded nucleic acid;

removing the unreacted labeled single-stranded nucleic acid, and

measuring fluorescence intensity of each spot of
the probe array to obtain a first template pattern
showing a relationship between location of the probes
and fluorescent characteristics;

(c) analyzing the first template pattern to locate probes and to calculate a mean value of fluorescence intensities (F_i) of the double-stranded nucleic acids having i of mismatched base pairs, where i is an integer not less than 1;

(d) calculating a difference ($F_{1, 0}$) between the fluorescence intensity of the fully complementary double-stranded nucleic acid without mismatch (F_0) and the mean value of the fluorescence intensities of the double-stranded nucleic acids having one-base mismatch (F_1), further calculating a difference ($F_{i+1, i}$) between a fluorescence intensity of a double-stranded nucleic acid having ($i+1$) base mismatches (F_{i+1}) and a fluorescence intensity of a double-stranded nucleic acid having i -base mismatches (F_i), and identifying i being $F_{i+1}, i << F_i, i-1$;

(e) assuming a target DNA which base sequence is complementary to the second probe sequence, then obtaining the second template pattern formed by the probe position where the number of mismatched base pairs to the target having the complementary sequence to the second probe sequence is not more than i;

(f) performing the same operation as the step (e) for each of remaining single-stranded nucleic acid probes using a third to a nth single-stranded nucleic acid, and obtaining template patterns of No. 3 to No. n showing a relationship between location and fluorescent characteristics of the probes, wherein the template patterns are formed from the positions of the probes having a base sequence that forms mismatched base pairs in a number not more than i;

(g) performing the same operation as the step (b) using a sample containing the target single-stranded nucleic acid of unknown base sequence to obtain a sample pattern showing relationship between a position and fluorescent characteristics; and

(h) comparing the sample pattern obtained in the step (g) with n pieces of template patterns obtained in the steps (b), (c) and (e), to identify a template pattern showing essentially the same pattern as the sample pattern and identifying the base sequence of the single-stranded nucleic acid used for the preparation of the identified template pattern as the unknown base

sequence of the target single-stranded nucleic acid.

5 3. The method according to claim 2, wherein the step (g) further comprises the substep of obtaining a two-valued pattern of the fluorescence intensity by using the threshold fluorescence intensity F_i .

10 4. The method according to claim 2, wherein the length of the probe is 8 mer to 30 mer.

15 5. The method according to claim 4, wherein the length of the probe is 12 mer to 25 mer.

20 6. The method according to claim 2, wherein the number of the mismatched base pairs (i) is 1.

25 7. The method according to claim 1, wherein in the steps (b), (c) and (d), the probes in the probe array are heat-denatured in a solution containing the single-stranded nucleic acid, and cooled to a temperature suitable for a double-stranded formation reaction while the substrate is soaked in the solution.

30 8. The method according to claim 7, wherein the length of the single-stranded nucleic acid probe is 18 mer, the temperature for performing the heat denaturation is $70^\circ C$ or more, the temperature for the

double-strand formation reaction is 40°C or more, and
100 mM sodium chloride is contained in the sample
solution at that time.